

EARLY DEVELOPMENT OF *LIMNODYNASTES TERRAEREGINAE* AND *L. FLETCHERI* (ANURA: LEPTODACTYLIDAE: LIMNODYNASTINAE)

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Summary

DAVIES, M. (1992) Early development of *Limnodynastes terraereginae* and *L. fletcheri* (Anura: Leptodactylidae: Limnodynastinae). *Trans. R. Soc. S. Aust.* 116(4), 117-122, 30 November, 1992.

The development biology of *Limnodynastes terraereginae* and *L. fletcheri* is described. Life history data generally conform with those of congeners in the same species groups. Tadpoles show lentic adaptations and have a generalized body form. At 30°C, *L. terraereginae* completed its development in 71 days and *L. fletcheri* in 60 days.

KEY WORDS: Anuran larvae, development, *Limnodynastes terraereginae*, *Limnodynastes fletcheri*, Limnodynastinae, tadpoles, foam nests.

Introduction

Limnodynastes Fitzinger comprises 12 species (Tyler 1992) that can be grouped into three morphotypes: burrowing species with short hind limbs and a rotund body, 29-46 mm in body length (two species: *L. ornatus* group), larger burrowing species (52-90 mm length) characterised by well-developed tibial glands (four species: *L. dorsalis* group) and more streamlined non-burrowing species found in marsh and flooded grassland, and ranging from 31-75 mm in body length (six species: *L. tasmaniensis* group) (Tyler *et al.* 1979).

Limnodynastes spp. lay foamy egg masses (but see Roberts & Seymour 1989), but the entire developmental biology has been described only for *L. ornatus* (Tyler *et al.* 1983). Watson & Martin (1973) described tadpoles of *L. interioris*, one of the large burrowing species and examined larvae of *L. dumerilii*, *L. terraereginae*, *L. peroni*, *L. salmini*, *L. tasmaniensis* and *L. fletcheri* to permit a generic definition. However, these authors did not describe these larvae specifically, nor did they provide complete developmental data for *L. interioris*.

Martin (1965) described tadpoles and early stages of *L. dumerilii*, *L. peroni* and *L. tasmaniensis* whilst Tyler *et al.* (1983) described tadpoles of *L. convexiusculus*. Here I describe the developmental biology of *Limnodynastes terraereginae* Fry, (*L. dorsalis* group) and that of *L. fletcheri* Boulenger, (*L. tasmaniensis* group).

Materials and Methods

Spawn of *L. terraereginae* was collected 7 km SW Pentland, Qld and transported to the University of Adelaide after eight days, where it was reared at 30 ± 0.5°C in aerated dechlorinated tapwater contained in glass aquaria 25 × 25 × 8 cm.

Spawn of *L. fletcheri* was obtained from individual adults from Deniliquin, N.S.W., that spawned in the laboratory. This material was reared in the same manner.

Developing larvae were fed lightly-boiled, organically-grown mignonette lettuce leaves supplemented with SERA bioflakes pond fish food. Water and food were changed daily.

Samples of eggs, embryos and larvae were collected as shown in Tables 1 and 2 and preserved in Tyler's fixative (Tyler 1962). Cuts were made with a scalpel

TABLE 1. Dimensions of developmental stages of *Limnodynastes terraereginae*.

Age (approx. days, date)	Stage	Body Length \bar{x} , range in parentheses	Total Length \bar{x} , range in parentheses	n
1 27.i.1991	20	2.37 (2.24-2.4)	5.24 (5.2-5.68)	5
4-17 28.i.1991-12.ii.1991	25	4.19 (2.48-7.2)	11.12 (7.12-19.52)	62
17-22 10.ii.1991-15.ii.1991	26	6.69 (5.6-7.2)	18.08 (15.84-19.52)	10
21-28 14.ii.1991-8.iii.1991	27	9.27 (7.04-11.97)	25.15 (18.4-32.13)	14
28 27.ii.1991	28	8.72	34.45	1
55 19.iii.1991	29	11.03 (10.5-11.55)	29.72 (29.4-30.03)	2
28-55 21.ii.1991-19.iii.1991	31	11.07 (8.72-12.45)	35.69 (34.03-38.18)	3
55 19.iii.1991	32	14.12 (13.7-14.53)	38.81 (38.18-39.43)	2
50-70 14.iii.1991-7.iv.1991	34	15.13 (14.53-15.8)	42.94 (41.5-44.6)	5
55-70 19.iii.1991-7.iv.1991	36	15.51 (14.0-16.5)	43.77 (39.5-46.7)	7
55-70 19.iii.1991-7.iv.1991	37	15.98 (14.0-17.6)	45.84 (41.7-49.7)	12

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in the presumptive region of the tibial gland of *L. terraereginae* in the metamorphosing specimens.

Measurements of developmental stages were made using an eyepiece micrometer or vernier calipers (reading to 0.05 mm). Illustrations were made with the aid of a Wild M8 stereodissecting microscope and camera lucida.

Developmental stages are those of Gosner (1960).

Results

Limnodynastes terraereginae

Spawn was collected from a heavily-vegetated, deep, roadside depression at approximately 0800 on 26.i.1991. Adults of *L. terraereginae*, had been heard calling at this site the previous night, just before midnight, under an isolated patch of vegetation. The foam nest was large, but an egg count was not obtained, nor were eggs preserved.

At 2130 on 28.i.1991, embryos were at stage 25 (Fig. 1). Adhesive glands were reduced to prominent pigment patches. The horny beak and one upper and one lower complete tooth rows were keratinized. The anal opening was median and the spiracle had formed. Embryos remained at stage 25 for a further 24 hr. Feeding had commenced (faeces were in the anal tube). Faint pigmentation was detectable on the adhesive glands. One complete and one divided upper and two complete lower labial tooth rows were keratinized.

Embryos sampled on 30.i.1991 and 1.ii.1991 remained at stage 25. Pigmentation on the adhesive glands was detectable although considerably reduced in the older embryos. Further keratinization of tooth rows was not apparent.

Adhesive gland pigmentation had vanished by 4.ii.1991, although embryos were still at stage 25. The body was more heavily pigmented together with the tail

musculature and a lighter dusting on the tail fins. A second divided upper labial tooth row was keratinized.

Embryos sampled on 7.ii.1991 remained at stage 25, the only change being an increase in pigmentation particularly on the tail fins. Measurements are given in Table 1.

A single stage 26 larva was sampled on 10.ii.1991 and a second on 12.ii.1991. Pigmentation had increased in these larvae. Stage 27 larvae sampled on 14.ii.1991 had strongly pigmented bodies, tail musculature and tail fins. By 15.ii.1991 stage 27 larvae had a further divided upper and a divided lower tooth row keratinized.

By 21.ii.1991, larvae had reached stage 28 and pigmentation of the body and upper tail musculature had darker pigment patches superimposed over the uniform background. Most of these pigment patches surrounded the neuromasts of the lateral line organs.

By 8.iii.1991, a single stage 31 larva had well-differentiated lateral line organs and the hind limb paddle was pigmented dorsally, extending along the mediolateral surface of the hind limb bud by stage 34. Larvae attained stage 37 by 14.iii.1991.

Measurements of all larvae sampled are given in Table 1.

A larva at stage 36 is shown in Fig. 2. The body is ovoid and widest behind the eyes. The snout is evenly rounded in dorsal and lateral views. The nares are dorsolateral and sessile, opening laterally. The eyes are lateral and relatively large. The spiracle is sinistral, short and ventrolateral with a small orifice directed posterodorsally. It is attached along its medial edge to the body of the larva. It is visible from above and is slightly tapered towards its orifice.

The anal tube is medial and opens at the extremity of the ventral fin. The tail fins are poorly arched and rounded terminally. The dorsal fin commences in the posterior 1/10 of the body, being deepest about halfway along its length. The tail musculature is thick, tapering to a subacuminate terminus.

Tadpoles are moderately heavily pigmented and chocolate markings usually surrounding neuromast cells of the lateral line are superimposed on the background pigmentation. The mouth is ventral and the oral disc is surrounded by lateral and posterior labial papillae interrupted by an anteromedial gap. There are five or six upper and three lower labial tooth rows. The first upper and second and third lower rows remain undivided. The horny beak is moderately robust. The oral disc of a larva at stage 36 is shown in Fig. 3. The first larva reached stage 42 on 2.iv.1991 and stage 46 on 7.iv.1991 having taken a total of 71 days to metamorphose from spawning. Froglets at stage 46 measured 18.00 mm S-V.

Supralabial glands were apparent at stage 42 and although not apparent externally, glandular tissue was detected by eye in cut skin in the region of the presumptive tibial gland.

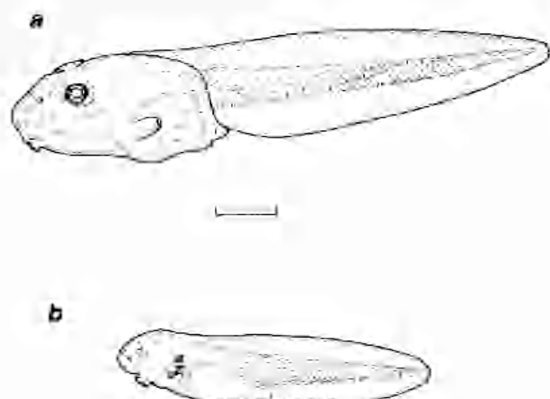


Fig. 1. Embryos of *Limnodynastes terraereginae*: a, Stage 25; b, Stage 20 of hatching. Scale bars = 1 mm.

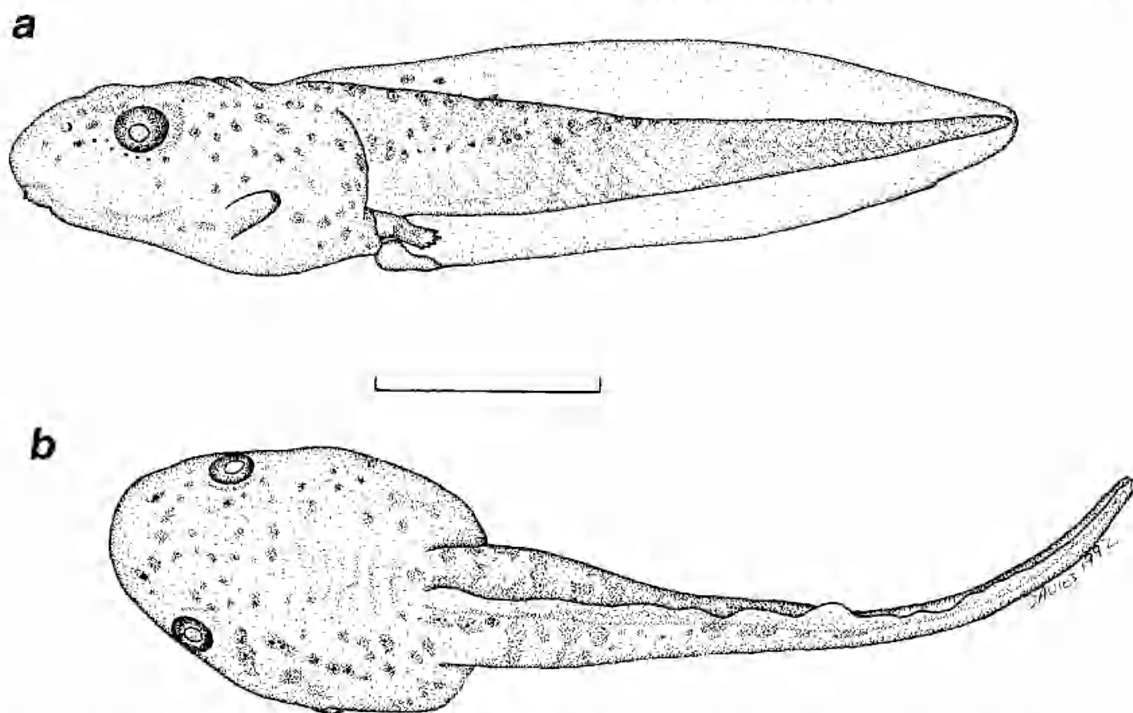


Fig. 2. Larva of *Limnodynastes terraereginae* at Stage 36: a, lateral view; b, dorsal view. Scale bar = 10 mm.

Limnodynastes fletcheri

Spawn was laid in the laboratory in a chamber of recyling, continuously-flowing water described by Chapman (1987). Two spawn clumps deposited overnight on 16/17.xi.1991 and 21/22.xi.1991

respectively were collected. Data are mostly derived from the first clump. Spawn was laid in a foamy nest and twelve eggs from the second clump had a mean diameter of 1.39 mm (range 1.28-1.56). Mean capsule diameter was 1.80 mm (range 1.72-1.88).

Within 24 hours the embryos had reached stage 18 (Fig. 4) and hatched at stage 19, 24 hr later on 20.xi.1991 (Fig. 4). Gills were poorly developed. The mouth was perforated but the nares were not. Tail musculature was poorly delineated.

At 48 hr, the gills had disappeared, but the embryo remained at stage 19.

Embryos reached stage 25 four days after hatching. The anus was median. Slight protrusions of the adhesive glands remained, the base speckled with pigmentation. Over the next three days, the adhesive glands disappeared. The anus moved from a median to a dextral position and the horny beak, together with firstly one and then two upper labial tooth rows and three lower tooth rows, keratinized.

Measurements of stage 25 embryos are given in Table 2 and one is illustrated in Fig. 4.

Stage 26 and 27 larvae were sampled on the seven days after hatching. By stage 27, all tooth rows were keratinized. The body of the larva was irregularly pigmented and a faint dusting of pigment was apparent on the dorsal tail musculature and tail fins.

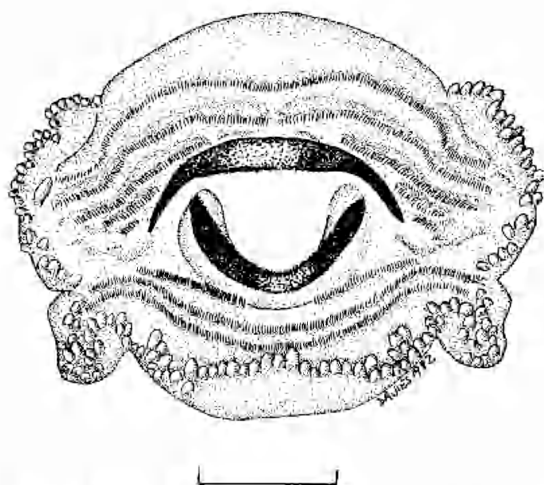


Fig. 3. Oral disc of a larva of *Limnodynastes terraereginae* at Stage 36. Scale bar = 1 mm.

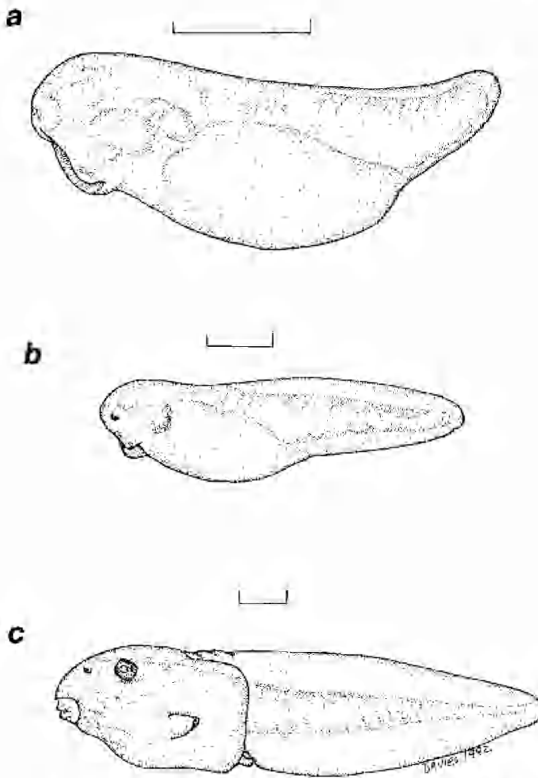


Fig. 4. Eubryos of *Limnodynastes fletcheri*: a. Stage 18; b. Stage 19 at hatching; c. Stage 25. Scale bars = 1 mm.

Larvae reached stage 30, 14 days after hatching. The anus was median and patches of chocolate pigmentation were appearing, superimposed over the background coloration of the body and tail. Measurements are given in Table 2.

Larvae reached stage 33 23 days after hatching and stage 35 after 27 days.

A larva at stage 37 is illustrated in Fig. 5. The body is ovoid and widest behind the eyes. The snout is evenly rounded in dorsal view and slightly truncated laterally. The nares are dorsolateral, not elevated, opening anterolaterally. The spiracle is sinistral and relatively short and is visible from above. It is attached to the body wall along its medial edge with the diameter of its orifice being less than the diameter of the tube. The spiracle tapers towards its orifice. The anal tube is median and opens along the ventral edge of the ventral tail fin. The tail fins are arched, the dorsal fin commencing in the posterior 1/10 of the body. Both are deepest approximately half way along their length. The tail fin is slightly rounded at its terminus. Tail musculature is moderately thick, tapering to a point posteriorly. Blotchy chocolate pigmentation on a cream background is located on the tail musculature with weaker melanin patches on the fins. The body is

TABLE 2. Dimensions of developmental stages of *Limnodynastes fletcheri*.

Age (approx. days, date)	Stage	Body Length \bar{x} , range in parentheses	Total Length \bar{x} , range in parentheses	n
6-11 23.xi.1991-28.xi.1991	25	4.97 (4.0-6.24)	12.49 (9.76-15.2)	27
10-17 27.xi.1991-4.xii.1991	26	7.01 (6.4-8.0)	18.07 (12.0-22.0)	6
10-26 27.xi.1991-13.xii.1991	27	7.25 (6.51-8.61)	19.83 (17.22-23.31)	12
17-26 4.xii.1991-13.xii.1991	28	9.79 (9.13-10.29)	26.48 (24.9-27.46)	3
26 13.xii.1991	29	10.49 (9.96-10.79)	27.73 (26.56-28.59)	4
17-26 4.xii.1991-13.xii.1991	30	11.91 (11.62-12.06)	30.71 (29.88-31.54)	3
47 15.i.1992	31	10.85 (10.3-11.4)	30.85 (30.2-31.5)	2
23-52 9.xii.1991-15.i.1992	32	13.83 (10.8-17.43)	36.96 (27.6-46.07)	18
26-47 13.xii.1991-15.i.1992	33	13.35 (12.0-14.8)	37.20 (34.4-40.26)	10
47-52 11.xii.1991-15.i.1992	34	14.36 (13.1-15.8)	38.83 (34.0-42.0)	14
47-52 8.i.1992-15.i.1992	35	16.23 (15.3-18.0)	44.23 (40.6-48.7)	4
52-70 8.i.1992-26.i.1992	36	17.08 (16.0-18.9)	48.32 (44.2-52.4)	5
70 26.i.1992	37	18.86 (17.5-20.5)	52.24 (48.8-56.0)	13
70 26.i.1992	38	19.0 (18.1-19.9)	57.1 (56.3-57.9)	2
70 26.i.1992	39	22.0	58.5	1
70 26.i.1992	40	24.2	68.5	3
65 21.i.1992	42	22.0	67.9	1
78 3.ii.1992	43	26.1	43.5	1
80 5.ii.1992	43	23.5	60.1	1
74 30.ii.1992	44	22.9	34.8	1
79 4.ii.1992	44	22.5	29.6	1
60 14.i.1992	46	21.2	-	1

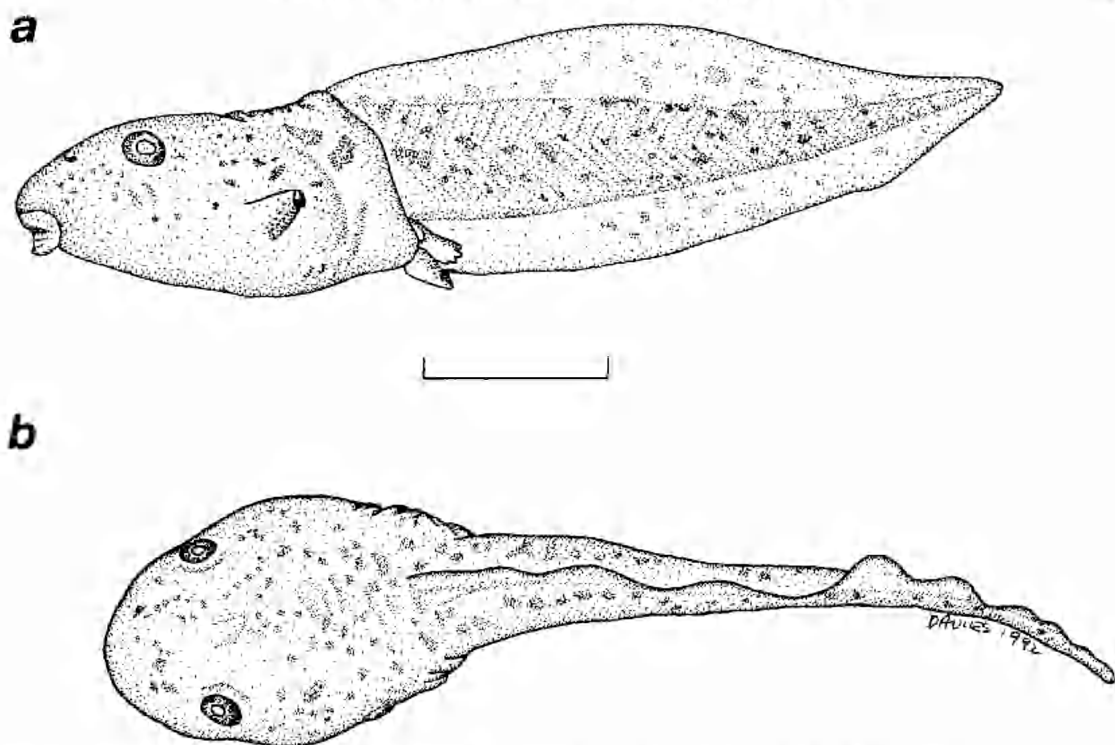


Fig. 5. Larva of *Limnodynastes fletcheri* at Stage 37: a. lateral view; b. dorsal view. Scale bar = 10 mm.

moderately pigmented with a paler cream posterodorsally. Chocolate freckles and smaller blotches are superimposed on the background pigmentation.

The mouth is anteroventral. The oral disc is surrounded laterally by a single row and posteriorly by a double row, of labial papillae. Papillae are absent anteromedially. There are three upper and three lower rows of labial teeth. Only those rows adjacent to the beak, which is moderately keratinized, are divided (Fig. 6).

Many of the oral discs examined were abnormal in development with split beaks and incomplete or distorted tooth rows.

Measurements of larvae are provided in Table 2.

By 12.i.1992 a larva had reached stage 42 and by 15.i.1992 it had reached stage 46, 60 days after spawning. Apart from a further three or four individuals, the remainder of the spawn clump did not metamorphose until about 30 days later, i.e., about 10.ii.1992.

Body length at metamorphosis was 21.2 mm.

Discussion

Of the tadpoles of large burrowing species of *Limnodynastes*, only those of *L. interioris* and *L.*

dumerilii have been described and illustrated (Watson & Martin 1973; Martin 1965). Larvae of *L. terraereginae* described here have a similar morphology to that of these species although the inner edge of the spiracle may be free in *L. dumerilii* and these larvae are usually darkly pigmented, with older tadpoles being generally lighter (Martin 1965). The

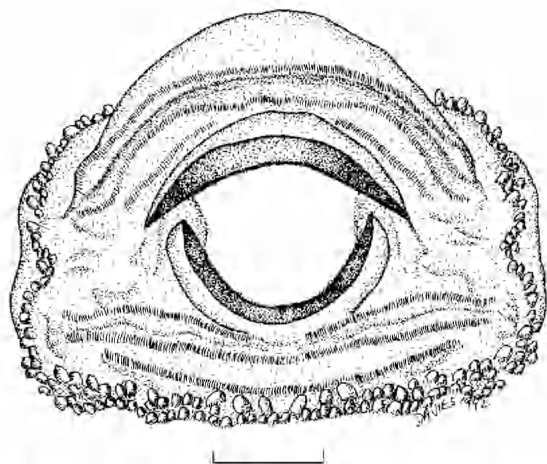


Fig. 6. Oral disc of larva of *Limnodynastes fletcheri* at Stage 37. Scale bar = 1 mm.

general lentic body form of the three species is similar (described as generalized by Watson & Martin 1973).

A tooth row formula of five or six upper and three lower labial tooth rows is consistent within the group and the pattern of labial papillae is common to the three species.

Of the marshy and flooded grassland species, larvae of *L. tasmaniensis* and *L. peroni* have been described by Martin (1965) and those of *L. convexiusculus* by Tyler *et al.* (1983).

Tail fins of these species and of *L. fletcheri* tend to be more strongly arched than those of the *L. dorsalis* group. *L. peroni* has four upper and three lower labial tooth rows, *L. tasmaniensis* have five upper and three lower rows and *L. convexiusculus* has five upper and three lower rows of labial teeth (Martin 1965; Tyler *et al.* 1983). Watson & Martin (1973) recorded at least four and usually five to six rows of upper labial teeth for *L. peroni*, *L. salmini*, *L. tasmaniensis* and *L. fletcheri*.

The presence of only three upper tooth rows in the *L. fletcheri* examined here may be a result of the high proportion of abnormal mouths in the two spawn clumps reared. Only one male was present in the colony of adults from which the spawn was obtained and given that larvae of *L. terraereginae* and *L. salmini* reared under identical conditions did not show the same

phenomenon, it is possible that the problem is a genetic one. Ridges lacking in teeth were apparent in the *L. fletcheri* tadpoles and it is possible under other circumstances that tooth rows form on these. It is known that larvae reared in the laboratory tend not to have tooth rows that are as well developed as those that are collected from the field but the deficiency in the rearing methods has not been identified (M. Davies, M. J. Tyler & G. F. Watson unpubl. data).

Whilst recognising the anomaly in the tooth row formula recorded for *L. fletcheri* here, larval characters are consistent with the species groupings based on adult morphology.

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